polymer followed the same course as that of the soybean polymer.

A larger amount of degraded stearyl vinyl ether polymer was prepared by passing oxygen through the polymer at 150°C. for 6 hrs. in the presence of 0.1% of cobalt naphthenate. The resulting product contained 31.4% acid calculated as stearic acid. A white crystalline acid was isolated from this mixture and chromatographed on a silicic acid column according to the procedure of Nijkamp (5). The single band obtained was eluted from the column. Material recovered from this band was placed on a second column with pure stearic acid. One band was obtained, thus confirming the identity of the acid from the degradation. A small amount of a water-soluble red oil was obtained from the degradation mixture which appeared to be a ketovinyl alcohol on the basis of infrared spectra and solubility.

The isolation of a C_{18} fatty acid and a material that appears to be a partially oxidized polyvinyl alcohol from oxidized soybean and stearyl polyvinyl ethers furnishes chemical evidence to support the proposed mechanism outlined by structures (I) and (II).

Summary

Vinyl ethers of stearyl, soybean, and linseed fatty alcohols have been prepared and polymerized in solution in hydrocarbon or chlorinated solvents at temperatures down to —30°C. with several Lewis-acid-type catalysts. Stearyl polyvinyl ether was a white, waxy solid melting at 44°–50°C. while soybean and linseed polyvinyl ethers were colorless, viscous liquids. Molecular weights of these polymers range from 1,500 to 15,000 and higher, depending on the conditions of polymerization.

Films of soybean and linseed polyvinyl ethers containing driers were cast from toluene solution. Hard films were obtained with cobalt drier by baking at 150°C. while softer films were obtained under these conditions when lead driers were used.

Soybean films containing cobalt drier and baked on Pyrex glass dissolved completely in 5% aqueous alkali. A fatty acid coresponding to the fatty alcohol side chain was isolated from this solution along with a material that appeared to be partially oxidized polyvinyl alcohol. Baked films of soybean polyvinyl ether with lead drier did not dissolve in alkali. Some improvement of alkali resistance was obtained with cobalt films by adding aromatic amines as antioxidants. Soybean polymer films containing cobalt and baked on soft glass or metal surfaces were resistant to 5% aqueous alkali for at least three days.

Soybean polyvinyl ether was emulsified with an equal weight of water, using ammonium salts of fatty acids as emulsifiers. Films were prepared from this emulsion that appeared to be continuous.

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Note on Acetone-Soluble Material in Cottonseed Meals

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FOR A NUMBER OF YEARS there has been research on inactivation of the toxic and growth-inhibiting material in cottonseed in order to extend the availability of commercial cottonseed meal for use in poultry and swine rations. The responsible substance has generally been considered to be the "free" or "unbound" gossypol remaining after processing of the seed and extraction of the oil from the meal. The considered opinion of most workers in poultry and swine nutrition is that reduction of "free" gossypol to 0.04% is sufficient to provide a meal which does not exhibit toxic or growth-inhibiting effects because of this substance when the meal is incorporated in poultry rations (10). A number of commercial and laboratory processes yield meals of 0.02 to 0.04% of "free" gossypol (9, 4), and some of these meals have been used in commercial broiler rations.

The problem of discoloration of eggs, when stored, from hens fed rations containing cottonseed meal is still unsolved. It is known that gossypol, incorporated in the diets of laying hens, causes development of an olive color in the yolks when the eggs are stored under commercial conditions (11, 12). The consensus of workers in this field is that meals containing 0.01% or more of "free" gossypol will cause the egg discoloration when used in unlimited amounts in poultry feed (3).

Efforts to eliminate completely the "free" gossypol by applying drastic mechanical action to break the pigment glands, followed by cooking to bind the liberated gossypol, have failed to produce meals containing less than the 0.02 to 0.04% of "free" gossypol (7). Reduction to values of 0.005 to 0.015% has been achieved in the laboratory by extraction with polar solvents (2, 5). None of these procedures has acquired commercial status up to the present time. Extraction with polar solvents, such as aqueous acetone or butanone, not only reduces the "free" gossypol to low values but also removes the materials responsible for egg discoloration This has been demonstrated in some physiological tests. The nature of this material therefore becomes a key to the development of methods of eliminating egg yolk discoloration.

"Free" gossypol is determined by extraction with

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aqueous acetone, followed by development of color with an amine, aniline, or p-anisidine. It has been assumed in this test that the colored material which is measured is either gossypol or gossypol-like pigments. This method has been very useful in determining the "free" gossypol in meals and has, as pointed out earlier, been invaluable in helping to establish the basis for production of meals that can be fed to broilers and swine. When however the point has been reached where meals have a low "free" gossypol content—low enough to be fed safely to swine and broilers but not low enough to be fed to laying hens—the question of the nature of the extractable material becomes important. We have found that not all of the aniline-reacting material extractable with aqueous acetone is gossypol. Small amounts —very small in terms of the total gossypol in the seed but possibly of importance in connection with the egg yolk color problem—of other materials are present.

"Free" gossypol is determined by the method of Pons *et al.* (8), which has been adopted as a tentative method by the American Oil Chemists' Society (1), and the use of the term "free" gossypol in this paper has reference to the data obtained by following that procedure. The term "free gossypol" in the A.O.C.S. method is defined as "free gossypol and gossypol-like substances." The method of analysis consists essentially of comminuting the cottonseed meal by shaking the sample with glass beads for 1 hr. in contact with 70% aqueous acetone. This action ruptures the remaining pigment glands, and the liberated gossypol dissolves in the solvent. The solution of "free" gossypol is separated from the meal particles by filtration, and the gossypol in solution is determined by developing the highly yollow-colored condensation product of gossypol with an aromatic primary amine, such as aniline or p-anisidine, followed by spectrophotometric determination of the gossypol by measurement of the increase in optical density of the solution at the appropriate wavelength.

When appreciable quantities of "free" gossypol are present, the absorption spectra are characteristic of gossypol. However when meals of low "free" gossypol content are analyzed by the procedure, characteristic gossypol and gossypol-amine condensation product spectra are not obtained.

One phase of a research program to produce meals which can be fed to laying hens is to determine the source and nature of this residual material that shows up as "free" gossypol by the A.O.C.S. analytical method. This is considered necessary in order to identfy the causative agent, or agents, of egg-yolk discoloration so that practical means can be found for their elimination from commercial cottonseed meals.

A procedure was developed to isolate the gossypol from the aqueous acetone extracts by transferring it to benzene. It was established that gossypol can be transferred consistently to the extent of 92% (see data in Table I). The extent of recovery of gossypol added to the 70% acetone extraction of cottonseed meal is illustrated by the following experiment:

TABLE I

Recovery of Pure Gossypol by Transfer to Benzene from 70% Acetone Soln.

mg. gossypol added to 25 ml. aqueous acetone	mg. gossypol recovered by benzene extraction
0.13	0.12
0.13	0.12
0.12	0.11

10.0 ml. of soln. of pure gossypol in 70% acetone, containing 0.051 mg. of gossypol, were added to 15.0 ml. of 70% acetone extract from commercial, prepress, solvent meal containing 0.022 mg. of gossypol. The gossypol was transferred to benzene by the procedure given below. Of the total of 0.073 mg. of gossypol added, 0.068 mg. or 93.5% was recovered.

Method for Transferring Gossypol from Aqueous, Acetone Sample Solution to Benzene.

A 0.5000-g. sample of the meal is agitated with 50.00 ml. of 70% aqueous acetone and glass beads in accordance with the A.O.C.S. procedure for "free" gossypol. To 25.00 ml. of the filtered sample solution are added 25.00 ml. of benzene in a separatory funnel. After shaking for two minutes, 25 ml. of water containing 0.05 to 0.10 g. of sodium hydrosulfite ($Na_2S_2O_4$) are added. The mixture is again shaken for two minutes. The last operation is performed thre more times, making a total of 100 ml. of added water. After the layers are allowed to separate, the upper (benzene) layer is filtered through paper into a small separatory funnel and washed three times with 10 ml. portions of water. The benzene layer is again filtered through paper.

Quantitative Determination of Gossypol in the Benzene Extract

The gossypol content of the benzene extract is determined by converting it into dianilinogossypol by heating with aniline. Spectrophotometric determination of the quantity of gossypol in the solution is made by measuring the absorptivity of the treated solution and appropriate blank solutions at a wavelength of 440 mμ in accordance with a previously described procedure (6). For greater precision a distribution factor of 100/92 indicated by the recovery experiment in Table I is applied.

Results obtained upon application of the above procedure to one commercial and three laboratory prepared meals of low (0.03 to 0.08%) "free" gossypol content show that the benzene extract contains the residual gossypol in a purified form, which gives the characteristic absorption spectra of gossypol and dianilinogossypol. This was also true of the benzene extracts of pure gossypol and of hexane-extracted raw cottonseed meats. Quantitative results are shown in Table II. It will be noted that the cooked cotton-

TABLE II

Recovery of Gossypol from Cottonseed Meats and Meals by Extraction with 70% Acetone Followed by Transfer from 70% Acetone to Benzene

Sample	% "Free" gossypol determined as dianilinogossypol in 70% acetone extract	% Gossypol determined as dianilinogossypol in benzene extract
Lab.-cooked whole meats	0.034	0.017
Lab.-cooked hexane extr. meats	0.046	0.028
Lab.-cooked hexane extr. meats	0.076	0.043
Commercial prepress-solv. extr. meal	0.041	0.015
Hexane extr. raw meats	1.30	1.30

seed meats and meals examined contained variable amounts of gossypol in the aggregate of "free" gossypol and gossypol-like substances." In each instance the quantity of gossypol was somewhat lower than the aggregate.

Summary

In connection with a study of methods of eliminating traces of "free" gossypol from cottonseed meals a procedure for isolation of gossypol from the 70% acetone extracts of the meals by transfer to benzene solution has ben developed. A procedure is also given for quantitative determination of the isolated gossypol. Analysis of four samples of cooked cottonseed meats and meal by the method showed that in each instance substances other than gossypol are measured by the A.O.C.S. method for "free" gossypol in meals

containing low concentrations of residual "free" gossypol. This procedure promises to provide another analytical tool for the study of residual material in processed cottonseed meal that causes egg discoloration when fed to laying hens.

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A Comparison of Alkylated Phenols as Antioxidants for Lard[1]

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ALTHOUGH CONSIDERABLE ATTENTION has been focused recently on the alkyl substituted phenols (hindered phenols), no systematic study relating their structure to antioxidant activity in fats has been published. Rosenwald *et al.* (1) has compared a number of alkyl phenols as antioxidants for gasoline in oxygen bomb tests. Wasson and Smith (2) recently reported a study of copper-catalyzed oxidation of lubricating oils to which various trialkyl phenols were added at 0.1% concentration (weight basis). The present paper reports the effects of some of the same and other compounds when equimolar quantities of them were added to lard.

Materials and Methods

Synthetic phenols were obtained from commercial sources whenever possible. All were recrystallized or redistilled before use. Four of the desired compounds which were not available commercially were synthesized in this laboratory. Sources and references are given in Table I for all except 2,4,6-tri-methylphenol, which was prepared as follows: 9.6 ml. conc. sulfuric acid were added to 20.0 g. mesitylene in a 125-ml. Erlenmeyer flask, and the mixture was held at 60°C. for 4 hrs. with frequent shaking. The supernatant liquid was decanted, the solid 2,4,6-trimethylbenzylsulfonic acid was dissolved in water, and 10% aqueous sodium chloride solution was added until the sodium salt of the sulfonic acid precipitated. This was filtered in a Buchner funnel, washed thoroughly with ether, and dried under reduced pressure; then 30 g. were added slowly to 84 g. of molten potassium hydroxide in a 500-ml. nickel crucible. The temperature was raised to 330°C. and held there for 10 min. The partly-cooled melt was poured into ice, the solution was acidified with hydrochloric acid and filtered. The crystalline product (2–3 g.) was recrystallized repeatedly from a methanol-ether solution until a constant melting point (68–69°C.) was obtained. Bruson and MacMullen reported a melting point of 69° for 2,4,6-tri-methylphenol (3).

The antioxidant activities of the various compounds were compared by addition of equivalent molar quantities to a lard of low antioxidant content, one micromol of antioxidant per gram of lard. The detailed procedure has been described elsewhere (4). All determinations were replicated: the active compounds five times, the inactive three times. In most cases replicates were started on different days.

Results and Discussion

Of the compounds tested, only those having an alkyl group in the *ortho*-position showed any antioxidant activity in lard (Table I). In all cases those having two alkyl group in the *ortho*-positions were more active than those having one, and an alkyl group in the *para*-position further increased the activity. Alkyl groups in the *meta*-position showed little or no influence on activity.

The nature of the alkyl group was less important than its position in most cases. However in the *ortho*-position *tertiary* butyl groups seemed to be most effective, and in the *para*-position methyl groups seemed most effective in enhancing antioxidant activity. Accordingly 2,6-di-*tert*-butyl-4-methyl phenol was the most active compound tested. This compound was more than twice as active as an equivalent molar quantity of catechol.

Nitro or halogen groups in *ortho* and *para* positions were not effective substitutes for alkyl groups since tribromophenol, triiodophenol, and picric acid showed no antioxidant effect. Evidently the function of the alkyl group is more fundamental than merely to provide a "steric hindrance" to the reactivity of the phenolic group. The antioxidant activity does not seem to bear an inverse relation to the acidity of the phenolic hydrogen or the inductive effects of substituent groups. Trinitro- and trihalogen phenols react readily with alkalies as do also the simple phenols. In contrast 2,4,6-trimethylphenol forms a sodium salt only on several hours of refluxing with alkali (3); 2,4,6-tri-*tert*-butylphenol does not form a sodium salt with alkali, and its solubility is diminished by the presence of alkali in alcohol (5).

While the inductive effect of the alkyl substituents

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